IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Timothy J. O'Brien	§	**ART UNIT:**
	§	**1643**
FILED: July 13, 2001	§	
	§	**EXAMINER:**
SERIAL NO.: 09/905,083	§	**Blanchard, D.J.**
	§	
FOR: Method of Inducing Immunity Against Stratum Corneum Chymotryptic Enzyme	§	**DOCKET:**
	§	**D6223CIP/C/D**

MS NON-FEE AMENDMENT
The Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, Timothy J. O'Brien, do hereby state as follows:

I am the inventor of the above-referenced patent application. I have read U.S. patent application serial no. 09/905,083 and I am aware of the content of the Office Action mailed July 25, 2005 and all prior art cited against the '083 application.

An issue relating to the patentability of a claimed method is the degree of enablement provided by the instant specification. The following data are presented as evidence of enablement commensurate with the scope of the claims:

Stratum corneum chymotryptic enzyme-5-13 (SCCE-5-13) peptide specific $CD8^+$ CTL recognition of CaOV3 ovarian tumor cells

SCCE-5-13 is the peptide of SEQ ID NO: 33 in the instant application and is derived from the signal peptide sequence of stratum corneum chymotryptic enzyme. This peptide binds HLA class I molecules as disclosed in the instant specification. Cytotoxic T cells specific for stratum corneum chymotryptic enzyme were derived by stimulation with dendritic cells pulsed with SCCE-5-13 peptide. Mature dendritic cells were loaded with peptide (2×10^6 dendritic cells with 50 μg/ml peptide in 1 ml serum-free AIM-V medium for 2h at 37^0C) and washed once prior to culture with 1×10^6/ml peripheral blood mononuclear cells in AIM-V or AIM-V plus 5% human AB serum. The peripheral blood mononuclear cell:dendritic cell ratio was between 20:1 and 30:1. After 7 days, responder T cells were restimulated with peptide-loaded, irradiated autologous dendritic cells or peripheral blood mononuclear cells at responder:stimulator ratios between 10:1 and 20:1 or 1:1 and 1:10 respectively. At this point, cultures were supplemented with recombinant human IL-2 (10-100 U/ml), and fed with 50-75% changes of fresh medium plus IL-2 every 2-4 days. T cell lines were established and maintained by peptide restimulation every 14-21 days. Responder $CD8^+$ T cells were purified by positive selection with anti-$CD8^+$-coupled magnetic beads (Dynal, Inc.) after the second or third antigen stimulation.

In the experiment depicted in Figure 1, SCCE-5-13 peptide-specific $CD8^+$ CTL recognition of CaOV3 ovarian tumor cells, CTL were derived by

stimulation with dendritic cells pulsed with SCCE peptide 5-13. Targets are autologous LCL loaded with 50 μg/ml peptide (◆), control LCL (✧), CaOV3 tumor cells (□), and CaOV3 tumor cells loaded with 50 μg/ml peptide(■). That is, LCL loaded with 50 μg/ml SCCE-5-13 peptide, CaOV3 tumor cells, CaOV3 tumor cells loaded with 50 μg/ml peptide and control LCL were incubated with the $CD8^+$ T cells at an Effector:target ratio between 2.5:1 and 10:1. Figure 1 shows that CaOv3 tumor cells are lysed to an equal extent whether or not they are pulsed with the SCCE 5-13 peptide. The CaOv3 ovarian tumor cells are also lysed to an extent that matches killing of a peptide-pulsed autologous lymphoblastoid cell line. This result provides strong evidence that the SCCE-5-13 peptide is naturally expressed, processed and presented by the ovarian tumor cells.

In conclusion, the results disclosed above show that SCCE-5-13 peptide, which is the peptide of SEQ ID NO: 33 in the instant application, can be used to stimulate stratum corneum chymotryptic enzyme specific CTLs. These CTLs were also shown to lyse CaOv3 ovarian tumor cells even when these cells were not pulsed with the peptide. This clearly indicates that even though peptide-5-13 is derived from the signal peptide of stratum corneum chymotryptic enzyme, it can still be used to generate a cytotoxic response against stratum corneum chymotryptic enzyme. Thus it is clear that peptides derived from the signal peptide of stratum corneum chymotryptic enzyme can be used in a method to generate an immune reponse against stratum corneum chymotryptic enzyme. These results further demonstrate that the computer program used in

the instant application correctly identified peptide-5-13 as an HLA class I molecule binding peptide.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code, and such willful false statement may jeopardize the validity of the application or patent issued thereon.

Date: Jan 18 2006

Timothy J. O'Brien